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## A comparison of using bulk and incremental isotopic analyses to establish weaning practices in the past

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### ABSTRACT

The use of incremental carbon and nitrogen isotopic analysis is gaining momentum as a way of establishing infant feeding practices in the past. Here we examine the differences in information gleaned through incremental isotopic techniques applied to individuals, relative to more commonly-used bulk isotopic sampling of a cross-section of a population. We use bulk sampling methods, which use bone collagen isotope values from multiple individuals, to construct Bayesian weaning curves for our samples. We then compare these results to individual weaning times established through incremental isotopic analysis of single deciduous teeth. Our results highlight that in contexts with high adult dietary variation it may not be possible to interpret weaning behaviour using cross-sectional techniques, and incremental isotopic analysis may be the only way of interpreting weaning behaviours. Our findings also suggest that cross-sectional analyses are the most useful way of creating population-scale interpretations of weaning behaviour within a sample. Incremental techniques, however, are necessary if we want to tell individual weaning stories and investigate the variation in infant-feeding present within the past.

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### KEYWORDS

Infant-feeding; carbon; nitrogen; isotopes




## Introduction

The investigation of breastfeeding and weaning practices in the past is significant for a number of reasons. Infant feeding practices have important implications for population dynamics (Bocquet-Appel 2002, 2011), female fertility (Jay 2009, Bentley, Paine, and Boldsen 2001) and infant and maternal health (Lawrence and Lawrence 2010, Stuart-Macadam and Dettwyler 1995).

Isotopic techniques have long been used to establish weaning times in past populations (Fogel, Tuross, and Owsley 1989, Wright and Schwarcz 1998, Schurr 1998). Weaning constitutes a dietary shift and is thus observable in changes to stable carbon and nitrogen ratios in bone collagen (Millard 2000, Richards, Mays, and Fuller 2002). While an infant is breastfeeding they are consuming their mother's protein, placing them a trophic level above their mother. This translates as a 2–3‰ increase in  $\delta^{15}\text{N}$  values (Fogel, Tuross, and Owsley 1989) and 1‰ increase in  $\delta^{13}\text{C}$  values relative to maternal values (Fuller et al. 2006). As supplementary foods are introduced and the infant begins weaning, their isotopic values gradually decrease until they align roughly with adult dietary values. In general, the investigation of weaning in archaeological

populations involves the bulk sampling of bone collagen from a sub-sample of differently aged infants and children within a cemetery population (reviewed in Tsutaya and Yoneda (2015)). The differences in isotopic values between different ages in this cross-sectional sample are then compared with each other, and adult dietary values from the same sample that act as a proxy for weaned diet (e.g. Jay et al. 2008). This allows bioarchaeologists to establish when weaning is generally initiated and completed in a population. Comparison can then be made with other groups or time periods to study changes to infant feeding practice.

Recently, however, incremental isotopic analyses have come to the fore as a method of establishing weaning trajectories on an individual level. In these techniques, tissues that grow at known rates and do not remodel through life are sampled in small increments to establish dietary changes through tissue formation (Montgomery et al. 2013, Beaumont et al. 2013, Eerkens, Berget, and Bartelink 2011, Henderson, Lee-Thorp, and Loe 2014, Burt and Garvie-Lok 2013). Dentine is one such tissue, and has a collagen component that can be analysed for carbon and nitrogen isotopic ratios just as it is in bone. Teeth that are

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forming throughout infancy and early childhood can, therefore, be examined to assess when weaning begins and the duration of the weaning process in each individual (Beaumont and Montgomery 2015, Beaumont et al. 2015).

Both of these isotopic sampling techniques are currently in use in archaeology, but as of yet no direct comparison between them using the same cemetery samples has been made to test whether or not they give the same information. Current literature describes the potentials and limitations of each method. Bulk sampling of infants and children allows general models to be built on a population-scale. Unlike incremental techniques, which require sampling of entire teeth, bulk sampling is less destructive as only small bone samples need to be taken. Sampling methods that use infant and child bone, however, also contain an inherent mortality bias (Wood et al. 1992). The infants analysed are the non-survivors and their mode of weaning may be directly linked to their lack of survival. Bulk sampling methods also create a generalised model of the weaning process for an entire cemetery sample, glossing over individual variation in favour of creating broad hypotheses (see Eerkens, Berget, and Bartelink 2011 for a review). In addition, there are issues with the use of bone samples to generate weaning times, as bone turns over throughout life resulting in isotopic values that represent diet over the months or years prior to death (Hedges et al. 2007, O'Connell et al. 2001). This should be accounted for when creating models to establish time of weaning (Tsutaya and Yoneda 2013).

Incremental techniques address some of these problems as they rely on a lack of turnover in the tissues analysed. This means that isotopic values more or less reflect diet at the time of formation (Beaumont and Montgomery 2015). Mortality bias may also be partly controlled for using incremental sampling. Individuals who survived childhood may be sampled alongside those who did not, as permanent first molars begin forming during early childhood and retain their weaning signal throughout life (Beaumont and Montgomery 2015, Beaumont et al. 2015, Hillson 1996). Using incremental techniques can also produce individual stories of weaning, allowing an assessment of the variability of infant-feeding practices in the past (Eerkens, Berget, and Bartelink 2011, Beaumont et al. 2015; King et al. Early view). Incremental techniques involve the cutting of teeth sampled into different increments which are analysed separately. This makes incremental analysis much more expensive and destructive to undertake, as a single individual may be represented by up to 20 samples, and may limit the number of individuals who can be studied using these methods. Finally, it is important to remember that, although incremental techniques give greater time resolution, the sampled dentinal increments cut across the geometry of tooth

development and therefore represent an average time period, rather than a specific point in time.

In this study we aimed to examine the differences in interpretation of the weaning process using incremental isotopic techniques relative to bulk isotopic sampling of multiple infants and children. In doing so, we highlight the different kinds of information each type of analysis provides, and situations in which incremental analysis may be the preferable analytical option. We use a sample from the Atacama Desert, northern Chile (1700BC – 1600AD), where adult diet is extremely heterogeneous to illustrate the potential pitfalls of bulk sampling in this kind of context.

## Materials and Methods

### Materials

The samples used in this project derive from the Arica region of the northern Atacama Desert, Northern Chile. They cover the agricultural portion of the archaeological sequence, dating from 1700BC – 1600AD (King et al. 2018 Early view-b, Arriaza 1995). This date range encompasses multiple archaeological phases from the incipient agricultural period (Formative), through the fully agricultural Middle (450-900AD) and Late-Intermediate (900-1450AD) Periods, to occupation of the Inka Empire in the Late Period (1450AD-1600AD). Samples from the Atacama Desert are generally at least partially mummified, and sampling restrictions put in place by the Museo San Miguel de Azapa, where the collections are housed, mean that the mummified individuals in the collections cannot be sampled for isotopic analysis. We were able to sample a statistically useful sample of 30 infants and children (< 15years) in total for bulk bone collagen analysis, and the deciduous dentine of 23 individuals for incremental isotopic analysis. We preferentially sampled the same individuals for incremental and bulk collagen analysis, but in many cases both tissues were not accessible in the same individual (paired samples  $n = 8$ ). Full details of the incremental sample are given in supplementary Table 3.

In our analyses we grouped individuals from the Formative –to Late Intermediate Periods ( $n = 20$ ) together, and considered the Late Period ( $n = 10$ ) separately. Though this wide temporal breadth would not be useful if we were making interpretations of past weaning behaviour over time, it is necessary in this study to ensure sample numbers are sufficient to allow generation of weaning curves. We consider the combination of samples into broader time periods viable because previous bulk isotopic analyses have highlighted a lack of significant isotopic differences between the early agricultural periods, and a continuity of population is assumed between the sites and

archaeological phases (Andrade et al. 2015, Watson et al. 2013, Sutter 2000, King et al. 2018). The Late Period sites analysed do, however, have a significantly different adult dietary baseline to those of other agricultural phases and are therefore considered separately. Adult bulk bone samples, used as a proxy for weaned diet also derive from our previous studies in the region (King et al. 2018), with means and standard deviations for each phase reported in supplementary Table 1.

Infant and child ageing was conducted primarily using dental development and eruption (AlQahtani, Hector, and Liversidge 2010) and epiphyseal fusion (Scheuer and Black 2004) for older children. Sex and age estimation of adults was conducted using established standards (Buikstra and Ubelaker 1994). Full details of the infant and child bone samples used in this study are given in supplementary Table 2.

We compare bulk infant and child bone collagen data with incremental isotopic profiles generated as part of our larger research project of infant and child weaning and dietary practices in the region (King et al. Early view). Deciduous and permanent first molars, which form from -0.2 years to 3.5 years, and 0.3 to 10 years respectively (Beaumont and Montgomery 2015, AlQahtani, Hector, and Liversidge 2010) were used.

Bone turnover processes mean that the isotopic values of bone collagen may represent a homogenised dietary signal. We intended to use dentinal collagen to assess whether the finer time-resolution possible in incremental sampling changes interpretations of weaning practices.

### Collagen preparation

Incremental sampling of dentine was conducted following method 2 of Beaumont et al. (2013). Teeth were half-sectioned longitudinally, enamel was removed using a diamond cutting disk, any secondary or tertiary dentine was removed using a dental burr (Beaumont et al. 2015). Half-sectioned teeth were demineralized in 0.5M HCl until the dentine was flexible. Dentine increments of 1 mm were cut using a sterilized surgical steel scalpel with a metal ruler for measurement, and placed into pre-weighed 1.5 ml microcentrifuge tubes.

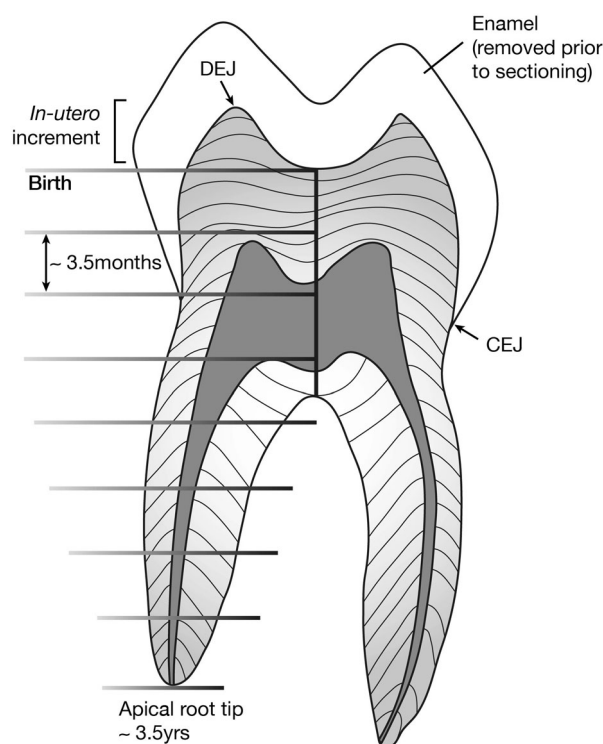
Both bulk bone collagen samples and dentine increments were prepared using a modified Longin Method (Longin 1971), detailed in King et al. (Early view-a, 2018). This involved demineralization in 0.5M HCl, gelatinization in a pH3 HCl solution at 75°C overnight, followed by lyophilization. Collagen quality was assessed following DeNiro (1985). Total organic carbon, total nitrogen content and stable isotope analysis of the samples was undertaken at Durham University using a Costech Elemental Analyser (ECS 4010)

connected to a Thermo Delta V Advantage isotope ratio mass spectrometer. Carbon isotope ratios were corrected for  $^{17}\text{O}$  contribution and reported in standard delta ( $\delta$ ) notation in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB). Isotopic accuracy was monitored through routine analyses of in-house standards, which were stringently calibrated against international standards (e.g., USGS 40, USGS 24, IAEA 600, IAEA N1, IAEA N2).

### Post-analysis processing of data

Time periods represented by increments were calculated using Beaumont and Montgomery (2015). This method takes into account the tooth type, growth rate, and any wear or root resorption. It acknowledges that the geometry of tooth formation is not horizontal, and that horizontal sampling does cut across different formation stages. This means that increment formation times are not precise, but by using the same technique for each individual analysed we are comparing the same developmental stage in each of them (Beaumont and Montgomery 2015). The incremental sampling method and timings represented by increments for a deciduous molar are given schematically in Figure 1.

The initiation of weaning ( $t_1$ ), complete cessation of breastfeeding ( $t_2$ ), generation of Bayesian weaning curves from bulk samples, and joint probabilities (the probabilities that both  $t_1$  and  $t_2$  lie within the stated



**Figure 1.** Schematic diagram showing horizontal incremental sampling of a deciduous molar with reference to the geometry of tooth growth, and the relative timings of increment formation.

ranges) were calculated using the WARN package in R (Tsutaya and Yoneda 2013).

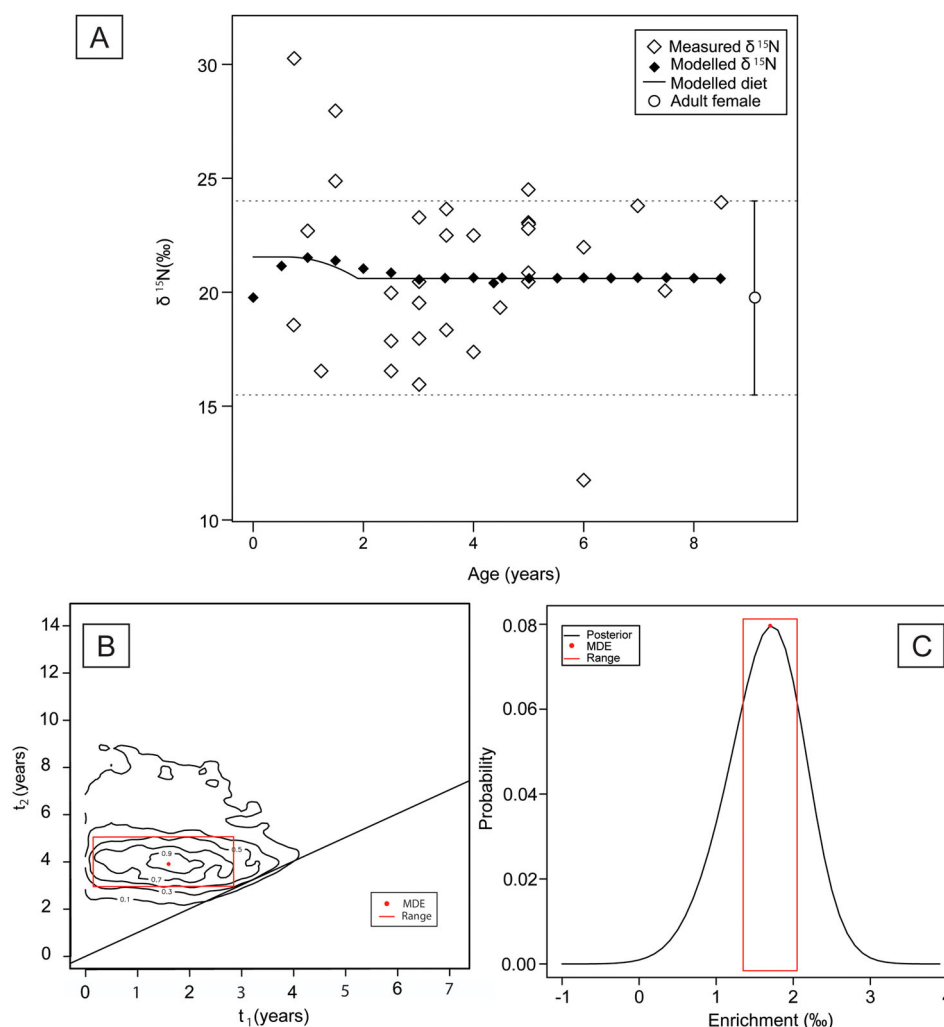
## Results

### Formative, Middle and Late-Intermediate comparison

In our sample, bulk sampling restrictions mean that weaning curves were generated using a relatively small number of samples. This, however, represents the unavoidable reality of sampling from archaeological contexts. In the early agricultural periods (Formative to Late Intermediate), bulk cross-sectional analytical methods cannot define a weaning curve for the sample. Bayesian curves should only be calculated for samples where a weaning curve is apparent from the raw data (Tsutaya and Yoneda 2013). Figure 2A illustrates that the raw data from these periods is extremely variable and it is difficult to imagine an expected weaning curve. In fact, some older individuals in this sample have higher  $\delta^{15}\text{N}$  values than some of the

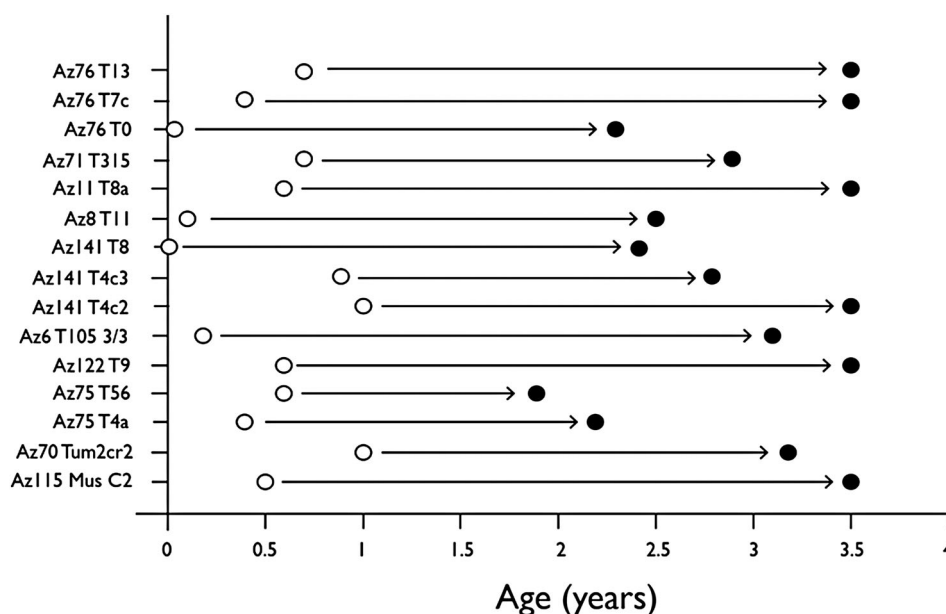
youngest individuals. If we ignore the pre-requisites of Bayesian analysis and force-fit a curve to our data, it seems to hint that a trophic level shift is barely occurring (Figure 2A). Maternal-infant enrichment is calculated as between 1–2‰ (Figure 2C), rather than the expected 3‰ characteristic of a trophic level shift (Fogel, Tuross, and Owsley 1989). WARN modelling estimates that weaning begins at 1.6 years of age ( $t_1$ ) and is complete by 3.9 years of age ( $t_2$ ). The ranges for  $t_1$  and  $t_2$  are 0.2–2.8 years and 3.0–5.0 years of age respectively, and the calculated joint probability that both  $t_1$  and  $t_2$  lie within the ranges is 0.512. The long time period and wide confidence intervals associated with this model reflect the high level of variation in isotopic results. This variability in infant and child bulk collagen results is likely linked to high adult dietary heterogeneity (King et al. Early view; 2018) transferred to the infants through breastmilk, and reflected in maternal choices of weaning foods.

Incremental isotopic results for each individual are given as supplementary Figure S1. Figure 3 summarises these results, showing the point at which weaning is



**Figure 2.** (A) Changes in nitrogen isotope values by subadult age (Formative – Late Intermediate Periods) generated using the WARN package. Dotted lines = 2 SD from female mean. (B) Contour lines showing the posterior probability for the combination of possible weaning ages MDE = maximum density estimate i.e. best estimate of mean weaning time. (C) distribution of posterior probabilities for maternal-infant tissue enrichment.





**Figure 3.** Showing the point at which  $\delta^{15}\text{N}$  values begin to decrease (white circles) and timing of when they become consistently 2-3‰ lower (black circles).

inferred to have begun, and when breastmilk no longer forms a significant portion of the diet in each of the individuals. In these periods, most individuals began weaning between six months and one year of age, and finished weaning between 2.5 and 3.5 years. The weaned values in our incremental results fit within the range of bone values in Figure 1. Individual profiles, however, show that weaning involves the expected 3-5‰ decrease in  $\delta^{15}\text{N}$  values, rather than the 1-2‰ enrichment calculated by the WARN model. The timings of weaning initiation and completion seen within our incremental results also do not align with the WARN model. The maternal dietary variability within this time period means that pinpointing the time at which weaning both begins and ends using bulk bone sampling is extremely difficult.

### Late Period comparison

Bulk isotopic results from the Late Period, however, do conform to a normal weaning curve (Figure 4A). Statistical analysis of these Late Period data using WARN indicates that the sample begins weaning at 1.1 years of age ( $t_1$ ), with weaning complete by 2.3 years of age ( $t_2$ ). The target ranges for  $t_1$  and  $t_2$  are 0.7-1.8 years and 1.9-2.7 years of age, respectively, and the calculated joint probability for the ranges is 0.534. The maternal-infant tissue enrichment in this phase aligns with the expected trophic level difference, lying around 3‰.

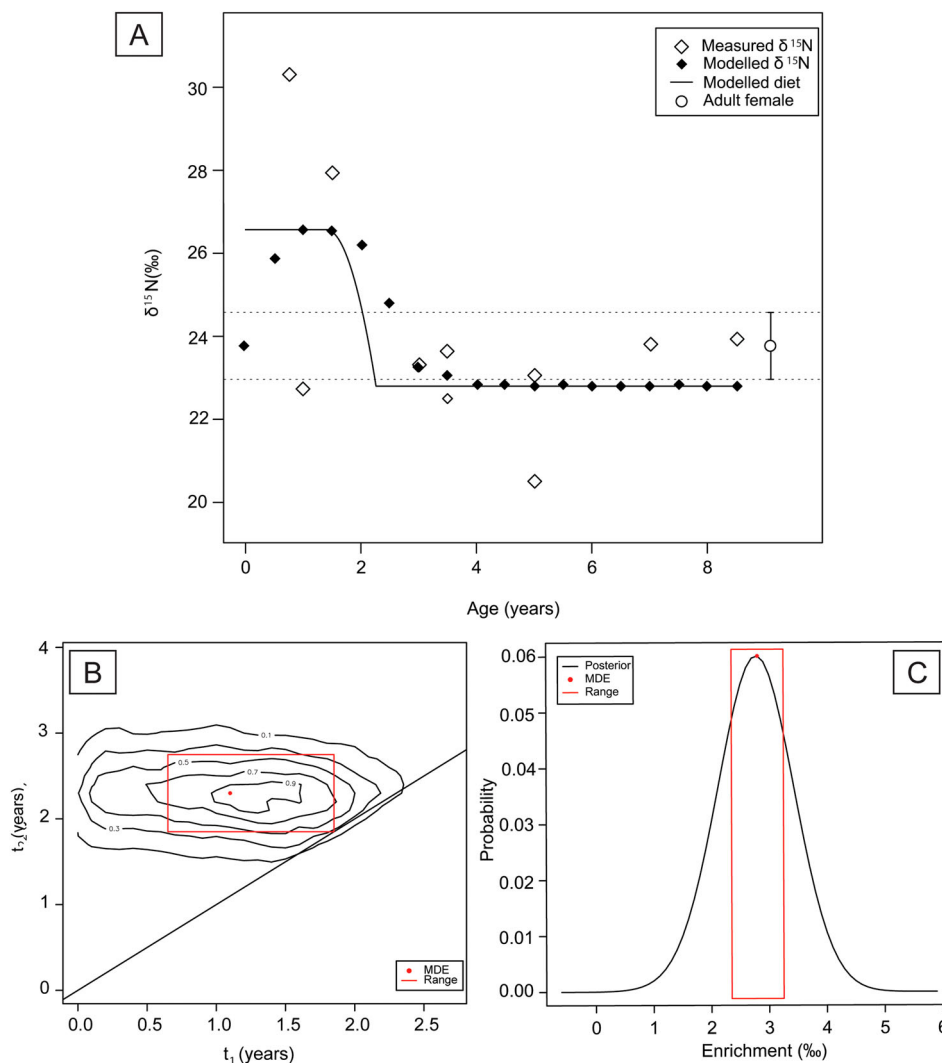
Data from incremental analyses, however, illustrates the variation in weaning experience present within the phase. This is not shown by the single time range given by bulk analysis. Figure 5 gives incremental isotopic profiles generated from Late Period individuals, and

highlights that infant feeding practice is non-uniform during this phase. While Cam9 T9 completes weaning at almost exactly the time predicted by the WARN model (2.5 years), Cam9 T27, Cam9 T53 and Cam9 T54c1 complete the weaning process later in life, at around 3 years of age. Cam9 T32c1 has  $\delta^{15}\text{N}$  values that remain unchanged from birth to time of death (2.5 years). This pattern could be interpreted as indicating that they were never breastfed, or that other processes such as physiological stress are also affecting  $\delta^{15}\text{N}$  values masking the expected weaning signal. Either way, this individual clearly does not fit the WARN model.

### Discussion

This study highlights the advantages of an incremental approach to isotopic sampling, particularly in contexts with high adult dietary variability. In our early agricultural sample baseline dietary variability prevents the generation of a weaning curve using a bulk bone sampling approach. Incremental isotopic analysis, on the other hand, does allow the investigation of weaning practices despite maternal dietary variability. This is possible because the technique uses the individual's own values to establish the point at which  $\delta^{15}\text{N}$  values begin to decrease (when weaning is initiated) and have decreased by one trophic level (2-3‰), signifying the cessation of breastfeeding. This analysis highlights that, when there is substantial adult dietary variability, incremental sampling may be the only way to create interpretations of weaning behaviour.

In addition, incremental isotopic analysis allows the characterisation of individual variation in infant-feeding practices within the population.



**Figure 4.** (A) Changes in nitrogen isotope values by subadult age (Late Periods) generated using the WARN package. Dotted lines = 2 SD from female mean. (B) Contour lines showing the posterior probability for the combination of possible weaning ages MDE = maximum density estimate, i.e. best estimate of mean weaning time. (C) distribution of posterior probabilities for maternal-infant tissue enrichment.

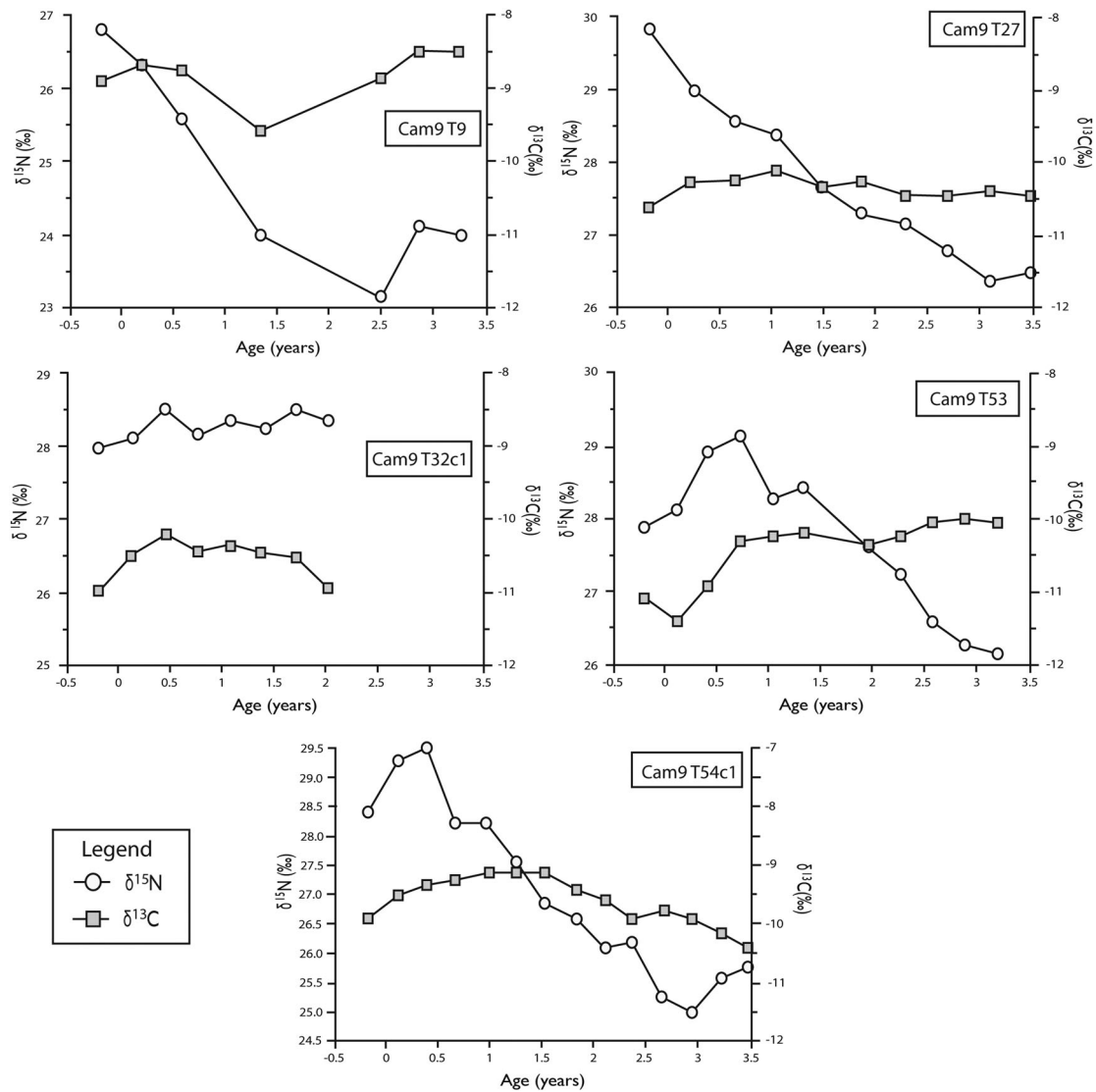
Although bulk bone sampling methods do allow modelling of general weaning behaviour (as with our Late Period samples), within any population there will be behavioural variation and not all individuals will follow the modelled curve (Kendall 2016). Bioarchaeologists are interested in both individual life stories and general trends over time and space. Incremental techniques highlight the variation in infant-feeding practices present within a sample. This is useful for more nuanced interpretations of dietary change and weaning histories.

#### **Other physiological processes which affect isotopic values**

Although not the focus of this article, it is also increasingly apparent that  $\delta^{15}\text{N}$  values may not simply reflect diet, but also other factors such as physiological stress (Fuller et al. 2005, Mekota et al. 2006, Beaumont and Montgomery 2016, Beaumont et al. 2015, King et al.,

Early view) or speed of growth (Warinner and Tuross 2010). It is difficult to assess whether or not archaeological infant and child bone  $\delta^{15}\text{N}$  values reflect the weaning process, times of accelerated or reduced growth, or periods of physiological stress. Incremental techniques, however, have the potential to allow the disentangling of dietary change from these other factors (Beaumont et al. 2015). The finer time-resolution possible using incremental techniques means that individual variation relating to growth rate can be more easily identified (Fuller et al. 2006, Warinner and Tuross 2010). In addition, by assessing whether  $\delta^{15}\text{N}$  values are higher than expected during breastfeeding or whether  $\delta^{13}\text{C}$  value changes echo those in the  $\delta^{15}\text{N}$  values (as expected in weaning), we may be able to identify isotopic variations that relate to stress rather than infant-feeding practices (Beaumont and Montgomery 2016). This means that incremental techniques may prove useful in quantifying the effects of stress on bone values used for cross-sectional sampling.





**Figure 5.** Incremental isotopic profiles from Late Period individuals analysed as part of this study. Midpoint ages represented by each increment have been calculated using Beaumont and Montgomery (2015).

### Limitations of incremental sampling

This study provides a foundation for understanding the different types of data that can be obtained using different isotopic methods. Although incremental sampling is useful in revealing the complexity of behaviours in the past, the use of incremental techniques alone has its limitations. By placing individual trajectories at the forefront of interpretation we are in danger of over-emphasising idiosyncratic weaning behaviour in the past. Additionally, the number of samples required from a single individual usually means that the researcher is restricted to analysing only a small number of individuals per phase. This means that, although our results show that no two individuals seem to have followed the same infant-feeding pattern, the individuals analysed are unlikely to be exactly contemporaneous. What we interpret as idiosyncratic weaning behaviours may simply be temporal variation in resource availability, or cultural expectations. Alternatively, our sample may represent the most

heterogeneous individuals, with other non-analysed individuals having more similarities in weaning practice. Although it would have been ideal to sample both bulk bone collagen and dentine from the same individuals, this was not always possible in this study. The sampling of different individuals may have exaggerated the differences between bulk bone and dentinal weaning results.

We suggest that the most useful interpretations of the past might be made using both bulk sampling of large numbers of individuals supplemented with sub-sampling individuals for incremental analysis. It is likely that the findings of incremental techniques will generally agree with timings gleaned using bulk bone sampling in most archaeological contexts. The advent of incremental techniques does not, therefore, render cross-sectional methods obsolete. Instead the use of both sampling methods will allow broad-scale population behaviour to be interpreted without over-emphasising idiosyncratic individual choices. Incremental results may then be used to supplement bulk

results or paleopathological evidence by allowing description of the variation in weaning practices, and reconstruction of individual stories.

## Conclusion

This case study illustrates the uses of incremental results, particularly in contexts where adult dietary variation is high. Both methods of sampling, however, have their merits and technique choice should be based upon the anthropological questions of interest. Bulk sampling a cross-section of the cemetery population remains the most useful and cost-effective way of building general models of population weaning behaviour. Incremental techniques allow characterization of variation and may be helpful in addressing issues of interactions between infant-feeding practices and physiological stress. Used together, these two techniques allow the building of large-scale archaeological models while considering the unique lives of individuals within those populations.

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## Disclosure Statement

No potential conflict of interest was reported by the author(s).

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